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SULFATE SALINE WATER AND ITS INFLUENCE ON SELECTED AQUATIC VERTEBRATES

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ABSTRACT

Sulfate Saline Water and Its Influence
on Selected Aquatic Vertebrates

The successful reproduction and growth of fish in North Dakota's saline waters is determined in part by the apportionment of dissolved salts. Trout were acclimated for 1 month at 14°C in dilutions of natural saline water having sulfate ion levels of 2000, 4000, and 6000 mg/l. Survival of trout in the latter 2 media was poor. The addition of Ca^{2+} ions in the form of $CaCl_2$ at a rate sufficient to increase calcium levels to 0.5% of the total dissolved solids permitted survival of trout in the 6000 mg/l SO_4^{2-} medium. Trout acclimated to 6000 mg/l SO_4^{2-} + Ca^{2+} supplemented medium exhibited a decrease in branchial (Na^+ + K^+)-stimulated MgATPase activity and an increase in the plasma sodium ion concentration. These changes were significantly different at the 5% level of probability when compared to the trout acclimated to the 2000 mg/l SO_4^{2-} + Ca^{2+} medium. No significant difference in body weight, body length or blood hematocrit was associated with saline acclimation to the varied sulfate levels.

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KEYWORDS-- biological membranes/electrolytes/fish/*ion transport/physiological ecology/Rainbow trout/saline lakes/*salinity tolerance/sodium chloride/*sulfates.

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CONCLUSIONS

1. Sulfate saline water adversely affects the ability of trout to maintain ionic homeostasis by impairing the ability to actively excrete sodium ions.

2. Calcium supplementation of sulfate saline increases survivorship and aids in the maintenance of osmoregulatory capabilities of trout.

APPLICATION OF RESULTS

The results of the project will be useful to fish culturists wishing to improve the quality of sulfate saline water. The addition of calcium to sulfate salines would make this water resource available for use to supplement water of higher quality, but lower availability. Furthermore, the results support the contention that water quality is determined by the type and apportionment of dissolved salts rather than simply their total quantity. As sulfate levels in surface water increase, the ability of fish to maintain their osmoregulatory capabilities decreases. The decrease may be associated with the inverse relationship between sulfate and calcium concentrations in sulfate saline waters.

INTRODUCTION

Useful rule-of-thumb standards of water quality have been developed based upon a surface water's total dissolved solids (TDS) or electrical conductivity. Rule-of-thumb standards, while capable of predicting the suitability of water for fisheries purposes, do not provide information on why fish do not survive in some saline lakes, or on how unsuitable saline lakes might be manipulated so as to enhance their value to sport fisheries.

North Dakota's prairie pothole lakes, estimated to cover 1.5 million acres, represent an enormous water resource. However, many of these lakes are saline. Lakes in Kidder County (Held, 1971) vary in salinity from 524 to 24000 mg/l TDS. Implicit in the development of the present study was the idea that water quality is determined by the type and apportionment of dissolved salts, rather than simply the total quantity of salts. The sulfate ion in the Kidder County lakes represents from 17 to 60 percent of the TDS, while in sea water sulfate ions represent only 8% of the 34330 mg/l TDS. Sulfate ion concentrations may also be singled out as contributing to the degradation of water quality for the following reasons:

1. Sodium sulfate is more soluble than sodium carbonate (Mitten, Scott and Rosene, 1968). In North Dakota where annual rates of precipitation are less than the annual rates of evaporation (Bavendick, 1952), sulfates could continue to become concentrated after the concentration of carbonates had become stabilized due to precipitation of the excess salt. Furthermore, under freeze conditions, normally tolerable levels of sulfate might become intolerable.

2. The effects of high sulfate ion concentration on the water and mineral balance of fishes is largely unknown. In marine species, less than 20% of the divalent ions acquired through drinking are absorbed through the gut. These ions are actively secreted across the renal tubular epithelium (Hickman and Trump, 1969). Sulfate ions are believed to be relatively impermeable to fish, and sodium sulfate is used to provide an impermeant ion in short-term kinetic studies of ion transport (Motais, 1967; Garcia-Romeu and Maetz, 1964).

The mechanisms of osmotic and ionic regulation in euryhaline fishes

have been reviewed (Hoar and Randall, 1969; Motais and Garcia-Romeu, 1972). The importance of branchial ion excretion, mediated through the sodium plus potassium stimulated Mg-adenosine trophosphatase transport enzyme system ($Na^+ + K^+$-stimulated MgATPase) has been emphasized (Kamiya and Utida, 1969; Zaugg and McLain, 1970; Kamiya, 1972). However, studies have been limited to adaptational responses to "typical" aquatic environments such as fresh water, dilute sea water or full strength sea water. Little work has been devoted to the effects of sulfate ion concentration on the osmoregulatory capabilities of fish, and few data exist on the body fluid concentration of sulfate (Magnin, 1962; Urist and Van de Putte, 1967). The present project attempts to describe the effects of varied sulfate ion concentrations on the *in vitro* activity of the branchial ($Na^+ + K^+$)-stimulated MgATPase system, the ability to regulate plasma sodium ion concentration and the blood hematocrit of the rainbow trout, *Salmo gairdneri*.

The project was to have included field and laboratory studies. However, attempts to maintain trout in selected Kidder County lakes failed due to loss of stocks through heat death or by the inability to establish trout (North Alkaline Lake, see Held, 1971). Attempts to produce a completely artificial sulfate medium also failed. Tris-SO_4^{2-}, Tris (hydroxymethyl) aminomethane, was used in formulating the artificial pond water. However, within a week after introducing trout, the water became turbid and oxygen depleted. Finally, survivorship media containing 4000 and 6000 mg/l SO_4^{2-} was poor. The results presented in this report were derived from trout that appeared healthy and were continuing to accept food at the end of a 1-month period.

OBJECTIVES

The object of the project was to assess the effects of sulfate salinity on the osmoregulatory ability of selected aquatic vertebrates, namely fish species. As pointed out in the Introduction, the proposed field portion of the study did not yield useful data. However, the laboratory portion of the study fulfilled the project objectives and also provided information that may be useful modifying the quality of sulfate saline water.

PROCEDURES

Rainbow trout, Salmo gairdneri, 110 to 150 mm in standard length were acquired from the Dakota Trout Ranch, Carrington, North Dakota. The trout were maintained at 14°C in groups of 5 fish per 10 gallon aquarium. The aquarium water was mechanically filtered and aerated. Lake George water was diluted to provide sulfate ion concentrations of 2000, 4000 and 6000 mg/l (see Mossier, 1971 for detailed water chemistry). Survival of trout in the latter 2 media was poor, and calcium levels were manipulated to increase survivorship. $CaCl_2$ was added in amounts calculated to yield Ca^{2+} levels equal to 0.5% of the TDS. Trout were fed a daily ration of commercial trout meal. At the time of sacrifice, trout were lightly anesthetized in a freshly neutralized solution of MS-222 (ethyl m-amino-benzoate methane sulfonate) containing 80 mg/l MS-222 (Wedemeyer, 1970). Severance of the caudal peduncle provided free-flowing blood samples that were collected in heparinized hematocrit tubes. The blood samples were centrifuged, the hematocrit determined and the plasma separated and frozen for later analysis. Plasma sodium concentrations were determined by atomic absorption spectroscopy. Lake water and its dilutions were assayed for O_2 using the azide modification of the Winkler method, for total and

calcium hardness, and for alkalinity using standard methods (APHA, 1965). Sulfate was assayed using HACH SulfaVer IV reagent (HACH Chemical Company, Ames). Total *in vitro* branchial MgATPase activity was assayed in a 1 ml assay system containing 94 µM NaCl, 20 µM KCl, 3.5 µM $MgCl_2$, 3.0 µM Na_2ATP and 30 µM Histidine-Cl buffer, pH 7.1. Residual MgATPase activity was assayed in the same assay system to which was added 1 µM ouabain. $(Na^+ + K^+)$-stimulated MgATPase is equal to the Total MgATPase minus the Residual MgATPase. All assays were run in duplicate with appropriate controls. Incubations were for 30 minutes at 37°C. The rate of phosphate liberation was linear for at least 60 minutes. Phosphate was determined by the technique of Lowry and Lopez (1946), and protein by the Folin's technique of Lowry *et al.* (1951) using bovine serum albumin as standard. Analytical techniques were checked against a commercial serum standard, Moni-Trol I (Dade). The preparation of the gill tissue and the sodium iodide activation are described in Figure 1. Samples means were compared by means of a one-way analysis of variance, and individual differences were assessed using the Student-Newman-Keuls Test (Sokal and Rohlf, 1969; Rohlf and Sokal, 1969).

RESULTS

Body weight and standard length of trout acclimated for 1 month in medium containing 2000 mg/l SO_4^{2-}, and calcium supplemented media containing 2000 and 6000 mg/l SO_4^{2-} did not differ significantly at the time of sacrificing (Table 1). However, trout acclimated to the 6000 mg/l SO_4^{2-} + Ca^{2+} supplemented medium tended to be smaller, and the lack of statistical significance may have resulted from the wide scatter of points and the small sample size. It should be noted that repeated attempts to acclimate trout to a 6000 mg/l SO_4^{2-} medium failed, and that survival was only possible

with calcium supplementation.

Acclimation to sulfate saline media adversely affected the ability of trout to regulate plasma sodium ion concentrations (Table 2). Trout acclimated to 6000 mg/l SO_4^{2-} + Ca^{2+} medium exhibited an average 12% increase in plasma sodium compared to the 2000 mg/l SO_4^{2-} + Ca^{2+} group. The difference was statistically significant ($p < 0.05$). Trout acclimated to 2000 mg/l SO_4^{2-} exhibited an intermediate plasma sodium concentration. Average blood hematocrits were not significantly different in the three treatment groups, but tended to be higher in the 6000 mg/l SO_4^{2-} + Ca^{2+} group.

The inability of trout to maintain plasma sodium levels when acclimated to high SO_4^{2-} levels is also indicated by their lower branchial (Na^+ + K^+)-stimulated MgATPase activity (Table 3). Acclimation to sulfate saline did not affect in vitro MgATPase activity, but resulted in a 24 and 62 percent decrease in activity of the 2000 mg/l SO_4^{2-} and 6000 mg/l SO_4^{2-} + Ca^{2+} groups when compared to the 2000 mg/l SO_4^{2-} + Ca^{2+} group (Table 3).

Acclimation to sulfate saline media reduce the trout's ability to maintain homeostasis in regard to plasma sodium (Table 2), and the reduced regulatory capability is apparently associated with a decreased ability to actively extrude sodium ions (Table 3). It is interesting to note that calcium supplementation of the lowest SO_4^{2-} treatment group apparently enabled the trout to maintain plasma sodium levels essentially identical to those reported by Huggel, Kleinhaus and Hamzehpour (1963) and only slightly less than the values reported by Holmes and McBean (1963) and Fromm (1963).

The apparent ability of calcium supplementation to improve survivorship and aid in the maintenance of osmoregulatory capabilities may be

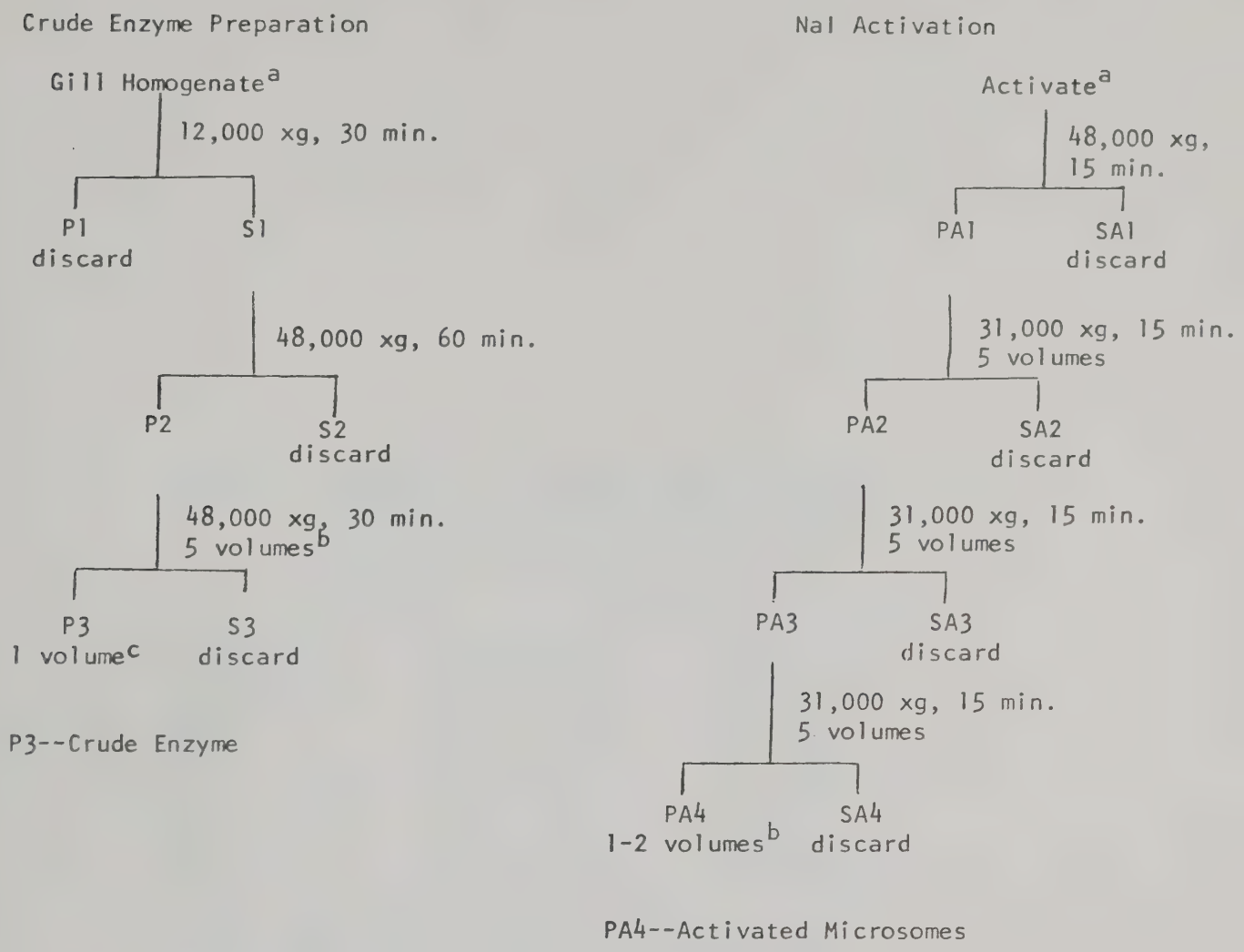
related to the ion's ability to stabilize cell membranes. Potts and Fleming (1971) demonstrated that reducing the calcium content of fresh water from 1 to 0.1 mM/l results in a more than 50% increase in the rate of sodium turnover in the euryhaline Fundulus kansae. The effect is mediated through changes in the ionic and osmotic permeability of the fish (Evans, 1975). Calcium supplementation in the present study resulted in calcium levels of approximately 0.3 and 0.9 mM/l in the 2000 mg/l and 6000 mg/l SO_4^{2-} media, respectively. Calcium levels in the Kidder County lakes included in Held's study (1971) vary inversely with sulfate concentration and TDS. Biologically available calcium may be zero in the high sulfate lakes.

Table 1. Morphometric data on trout taken at time of sacrificing. Trout had been acclimated for 1 month to sulfate saline media. Mean ± Standard Error (Number).

Treatment mg/l SO_4^{2-}	Body Wt g	Std Length mm
2000	52 ± 3.4(5)	163 ± 2.5(5)
2000 + Ca^{2+a}	44 ± 9.1(5)	150 ± 11.7(5)
6000 + Ca^{2+a}	33 ± 4.2(5)	145 ± 9.9(5)

[a] $CaCl_2$ supplemented to yield Ca^{2+} levels equal to 0.5% of Total Dissolved Solids.

Figure 1. Scheme used in the preparation of trout gill tissue for the in vitro assay of $(Na^+ + K^+)$-stimulated ATPase.



[a]Tissue homogenized in 9 volumes 30 mM Histidine-Cl, pH 7.1, 5 mM EDTA (Hist.-EDTA). Homogenate filtered through cheese cloth. Homogenizer washed with 10 volumes of Hist-EDTA and washings were filtered. Final tissue dilution 1:20.

[b]Hist.-EDTA.

[c]30 mM Histidine-Cl buffer. pH 7.1 Freeze in liquid nitrogen.

[a]Crude microsomes were incubated with mixing for 30 minutes on ice in an equal volume of solution containing a final concentration of 2 M NaI, 5 mM $MgCl_2$, 3 mM Na_2ATP, 5 mM Na, EDTA, 25 mM 2-mercaptoethanol in 50 mM TRIS-Cl, pH 8.0 @ 4°C (Nakao et al, 1965).

The mixture was diluted with water to yield a Nalodide concentration < 0.8 mM and centrifuged.

[b]Activated microsomes were immediately assayed for enzyme activity.

Table 2. Plasma sodium concentrations and blood hematocrits of trout acclimated for 1 month to sulfate saline media. Mean ± Standard Error (Number).

Treatment mg/l SO_4^{2-}	Plasma Sodium mEq/l	Hematocrit %
2000	144 ± 5.0(4)	25 ± 3.5(5)
2000 + Ca^{2+}[a]	139 ± 4.7(5)	25 ± 2.2(5)
6000 + Ca^{2+}[a]	155 ± 3.5(5)[b]	32 ± 4.0(5)

[a] $CaCl_2$ supplemented to yield Ca^{2+} levels equal to 0.5% of Total Dissolved Solids.

[b] mean significantly different $p < 0.05$, from 2000 + Ca^{2+} treatment.

Table 3. MgATPase and $(Na^+ + K^+)$-stimulated MgATPase activity in branchial tissue of trout acclimated for 1 month to sulfate saline media. Mean ± Standard Error (Number).

Treatment mg/l SO_4^{2-}	MgATPase µM Pi/mg x hr[a]	$(Na^+ + K^+)$-stimulated MgATPase µM Pi/mg x hr[a]
2000	1.8 ± 0.44(5)	2.2 ± 0.47(5)
2000 + Ca^{2+}[b]	2.1 ± 0.53(5)	2.9 ± 0.57(5)
6000 + Ca^{2+}[b]	1.1 ± 0.62(5)	1.1 ± 0.15(5)[c]

[a]micromoles inorganic phosphate liberated per mg protein per hour at 37°C.

[b]$CaCl_2$ supplemented to yield Ca^{2+} levels equal to 0.5% of Total Dissolved solids.

[c]mean significantly different, $p < 0.05$, from 2000 mg/l SO_4^{2-} + Ca^{2+} treatment.

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